AD_____

Award Number: W81XWH-04-1-0244

TITLE: The Role of the ADAM-15 Disintegrin in E-Cadherin Proteolysis and Prostate Cancer Metastasis

PRINCIPAL INVESTIGATOR: Dr. Mark L. Day

CONTRACTING ORGANIZATION: University of Michigan
Ann Arbor, MI 48109-1274

REPORT DATE: February 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-02-2006	Annual	20 Jan 2005 – 19 Jan 2006

4. TITLE AND SUBTITLE

The Role of the ADAM-15 Disintegrin in E-Cadherin Proteolysis and Prostate Cancer Metastasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0244

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Mark L. Day

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Michigan
Ann Arbor, MI 48109-1274

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

Our laboratory has shown that ADAM15 is predominantly overexpressed in metastatic prostate cancer. Importantly, the chromosomal location of ADAM15 on 1q21, a region of specific and high-level amplification in metastatic prostate cancer, makes this disintegrin a strong candidate in the malignant progression of this disease. Our preliminary data strongly suggests aberrant function of this protein in the growth, angiogenesis and metastatic progression of prostate cancer. Previous research indicates functional roles of ADAM15 in vascular endothelial biology with clear implications in endothelial interaction and angiogenesis. However, the specific actions of ADAM15 in tumor epithelium and influences on neighboring endothelial cells in the tumor microenvironment have not been explored. The continuing studies, if successful, will not only confirm the role of ADAM15 in the malignant processes of prostate tumorigenesis and progression, but will justify future translational studies of ADAM15 as a direct therapeutic target for this disease.

15. SUBJECT TERMS
metastasis, prostate

16. SECURITY CLASSIFICATION OF:				18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	6	USAMRMC

Table of Contents

INTRODUCTION

A key determinant in the metastatic progression of prostate cancer is the dissociation of cancer cells from the primary tumor that may result from inadequate cell adhesion. In tumors of epithelial origin, the disruption of cellular adhesion appears to arise, in part, through alterations of the E-cadherin cell-adhesion system. In our original proposal we hypothesized that the disintegrin metalloproteinase, ADAM 15, is closely associated with the metastatic progression of prostate cancer and could possibly cleave E-cadherin into proteolytic fragments. Examination of both cDNA and tumor microarrays demonstrated increased expression of ADAM-15 in metastatic prostate cancer. It was also important to note that the chromosomal location for ADAM-15, on 1q21, is a region of specific high-level amplification in prostate cancer metastasis. Taken together, this information provided a compelling rationale for the proposed studies and supports our central hypothesis: ADAM-15 specifically targets the extracellular domain of E-cadherin and disrupts the adhesive integrity of epithelium during the metastatic progression of prostate cancer. Not only will the proposed studies address the functional role of ADAM-15 in the metastatic transformation of prostate epithelial cells; these results may also justify future studies pursuing ADAM-15 as a direct therapeutic target for metastatic prostate cancer.

BODY:

Over-expression of ADAM15 in minimally malignant prostate epithelial cells to determine if this over-expression cleaves E-cadherin and induces a malignant phenotype. The intent of aim 1 was to achieve stable high-level expression of ADAM15 in LNCaP cell line and determine if ADAM15 elevation induces E-cadherin cleavage as well as a malignant phenotype in this minimally malignant prostate cancer cell line. ADAM15 was tagged with GFP on its C-terminus and transfected into LNCaP cells. ADAM15-GFP over-expressing LNCaP cells were verified via western blotting and immunohistochemistry by our laboratory (Figure 1). The inactive precursor form of ADAM15 is a 110 kDa protein which is converted into the 90 kDa active form by the pro-protein convertase furin. We will use the stable LNCaP cell lines (LNCaP ADAM15-GFP) to perform cell motility, invasion and anchorage-independence assays to determine malignant potential of theses cell lines.

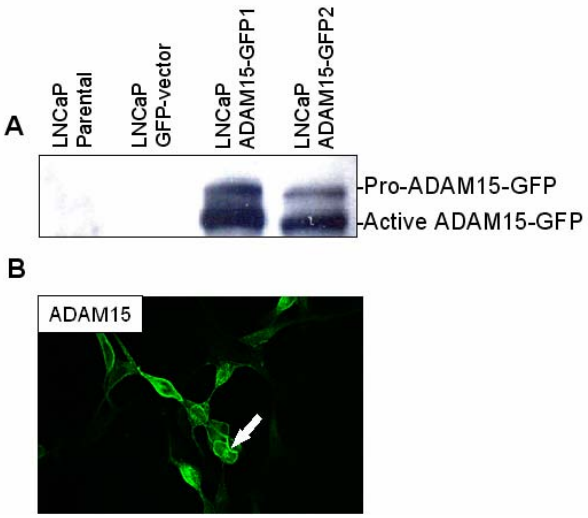

Figure 1. ADAM15-GFP LNCaP Cells. LNCaP cells were transfected with vector-GFP or ADAM15-GFP vector constructs. **(A)** Western blot showing the exogenous ADAM15-GFP in ADAM15-GFP transfected LNCaP cells. **(B)** Immunocytochemistry showing membranous staining (arrow) of ADAM15 in LNCaP cells.

To determine if ADAM15 knockdown reduces the cleavage of E-cadherin as well as the metastatic phenotype of highly aggressive prostate cancer cells. The results from aim 1 may indicate the ADAM15 promotes a malignant phenotype; however this does not confirm that ADAM15 is directly inducing this phenotype. Thus, the intent of this aim is to confirm that ADAM15 is specifically inducing the malignant phenotype seen by using reverse genetics. To accomplish this task, we will utilize small interfering (si)-RNA-mediated knockdown of ADAM15 using a short hairpin (sh)-RNA construct. We have permanently reduced ADAM15 expression in PC3 cells using ADAM15 siRNA oligos in a lentiviral system. To directly assess the contribution of ADAM15 to prostate tumorigenesis, we examined the ability of ADAM15 knock down cells (shA15PC-3^{luc}) cells to grow as subcutaneous tumor in male SCID mice. Following injection of shA15PC-3^{luc} cells and vector control (vecPC-3^{luc}) cells into both flanks of 5 mice per cell line, we could demonstrate a dramatic reduction in tumor growth starting at 3 weeks using the bioluminescence (**figure 2**). This experiment demonstrates the utility of our luciferase-based PC-3 tumor system and the ability to monitor tumor growth and progression in live animals.

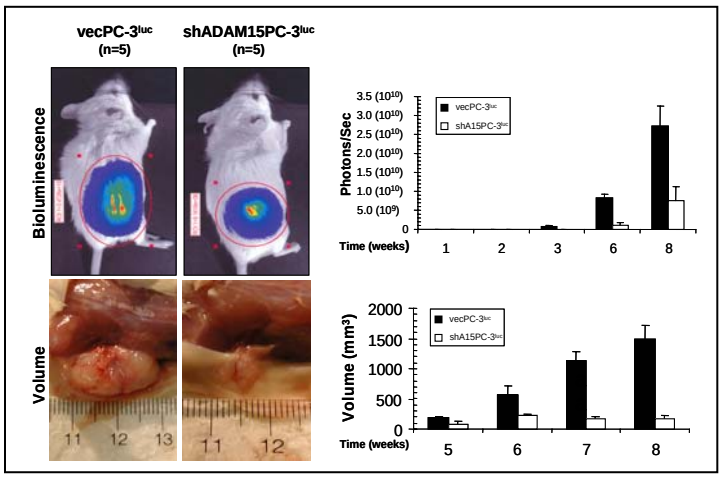

Figure 2. siRNA knock of ADAM15 inhibits in vivo PC-3 Tumor Growth. shA15PC-3^{luc} and vecPC-3^{luc} tumor growth was monitored over 8 weeks by bioluminescence and plotted as photons per second. At the end of 8 weeks the host animals were euthanized and the tumors excised for gross weight and volume measurements.

ACCOMPLISHMENTS:

1. ADAM15-GFP constructs were completed and successfully expressed in LNCAP cells.
2. Small interfering (si)-RNA-mediated knockdown of ADAM15 has been achieved as stable expression using viral vectors.
3. We have confirmed function of ADAM15 by demonstrating dramatic tumor reduction in vivo of ADAM15 knockdown PC-3 tumors.

REPORTABLE OUTCOMES:
We have created several cell lines that express ADAM15-GFP and have successfully knocked down ADAM15 expression in PC-3 cells. Initial in vivo results indicate that ADAM15 does indeed play a tumor promoting role in prostate cancer.

Abdo Najy who is a graduate student in my lab received a DOD predoctoral fellowship that will cover his salary and tuition through the remainder of this project.

We have a manuscript in press at Neoplasia that is the first comprehensive study of ADAM15 in prostate cancer. The DOD is cited as the funding source.

CONCLUSIONS:
In summary this study to date has yielded two important categories of information:

1. Necessary reagents for the remainder of the study are being generated and versified.
2. The clinical data examining the expression of ADAM15 in prostate cancer has been published and represents a solid translational platform with which to build the functional studies.